

What is claimed is:

- 1 1. A method for formulating an enzyme comprising:
2 obtaining at least one glucose oxidase gene;
3 creating at least one mutated glucose oxidase gene;
4 introducing each mutated glucose oxidase gene into separate expression vectors;
5 inserting the expression vectors into host organisms;
6 growing colonies of the host organisms; and
7 screening the colonies for desirable properties.
- 1 2. A method for formulating an enzyme according to claim 1, wherein screening the
2 colonies for desirable properties comprises:
3 determining whether the colonies contain active glucose oxidase; and
4 determining whether the colonies have peroxide resistant properties.
- 1 3. A method for formulating an enzyme according to claim 2, wherein screening the
2 colonies for desirable properties further comprises testing glucose oxidase from the colonies for
3 functionality.
- 1 4. A method for formulating an enzyme according to claim 2, wherein determining
2 whether the colonies have peroxide resistant properties is only performed if results of
3 determining whether the colonies contain active glucose oxidase are positive.
- 1 5. A method for formulating an enzyme according to claim 3, wherein testing
2 glucose oxidase from the colonies for functionality is only performed if results of determining

- 3 whether the colonies contain active glucose oxidase are positive and if results of determining
4 whether the colonies have peroxide resistant properties are positive.

1 6. A method for formulating an enzyme according to claim 2, wherein determining
2 whether the colonies have active glucose oxidase comprises employing a substance that changes
3 color in the presence of active glucose oxidase.

1 7. A method for formulating an enzyme according to claim 6, wherein the substance
2 is leuco-crystal-violet.

1 8. A method for formulating an enzyme according to claim 2, wherein determining
2 whether the colonies have active glucose oxidase comprises checking for fluorescence.

1 9. A method for formulating an enzyme according to claim 2, wherein determining
2 whether the colonies have peroxide resistant properties comprises:
3 incubating the colonies in peroxide; and
4 determining whether the colonies have active glucose oxidase after incubating the
5 colonies in peroxide.

1 10. A method for formulating an enzyme according to claim 2, wherein testing
2 glucose oxidase from the colonies for functionality comprises employing glucose oxidase from
3 the colonies in sensors.

1 11. A method for formulating an enzyme according to claim 10, wherein employing
2 glucose oxidase from the colonies in sensors comprises:
3 extracting glucose oxidase from the colonies;

4 immobilizing the glucose oxidase after extracting the glucose oxidase from the
5 colonies;
6 placing the immobilized glucose oxidase in a sensor; and
7 testing the sensor.

1 12. A method for formulating an enzyme according to claim 11, wherein extracting
2 glucose oxidase from the colonies comprises employing an ionic column to extract glucose
3 oxidase from the colonies.

13. A method for formulating an enzyme according to claim 11, wherein extracting glucose oxidase from the colonies comprises:

- removing the glucose oxidase from the colonies;
- purifying the glucose oxidase; and
- characterizing the glucose oxidase.

1 14. A method for formulating an enzyme according to claim 13, wherein removing
2 the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell
3 components.

1 15. A method for formulating an enzyme according to claim 14, wherein removing
2 the glucose oxidase from the colonies further comprises fractionating the cell components
3 employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

1 16. A method for formulating an enzyme according to claim 13, wherein removing
2 the glucose oxidase from the colonies comprises disrupting the colonies into cell components via
3 sonication.

1 17. A method for formulating an enzyme according to claim 16, wherein removing
2 the glucose oxidase from the colonies further comprises fractionating the cell components
3 employing centrifugation and differential solubility after disrupting the colonies via sonication.

1 18. A method for formulating an enzyme according to claim 13, wherein purifying
2 the glucose oxidase comprises purifying the glucose oxidase by employing chromatography
3 methods.

1 19. A method for formulating an enzyme according to claim 1, wherein the glucose
2 oxidase is obtained from an organism and wherein the organism is selected from a group
3 consisting of *Aspergillus Niger*, *Penicillium funiculosum*, *Saccharomyces cerevisiae*, and
4 *Escherichia Coli*.

1 20. A method for formulating an enzyme according to claim 1, wherein creating at
2 least one mutated glucose oxidase gene comprises employing polymerase chain reaction
3 techniques to create at least one mutated glucose oxidase gene.

1 21. A method for formulating an enzyme according to claim 1, wherein creating at
2 least one mutated glucose oxidase gene comprises employing error-prone polymerase chain
3 reaction techniques to create at least one mutated glucose oxidase gene.

1 22. A method for formulating an enzyme according to claim 1, wherein creating at
2 least one mutated glucose oxidase gene comprises employing gene shuffling techniques to create
3 at least one mutated glucose oxidase gene.

1 23. A method for formulating an enzyme according to claim 1, wherein the method
2 further comprises creating a next generation of mutated glucose oxidase genes after screening the
3 colonies for desirable properties.

1 24. A method for formulating an enzyme according to claim 23, wherein creating a
2 next generation of mutated glucose oxidase genes is repeated approximately 2 to 6 times.

1 25. An enzyme formulated according to the method of claim 1.

1 26. A method for formulating an enzyme comprising:
2 obtaining an organism with a glucose oxidase gene;
3 growing multiple colonies of the organism;
4 altering the environment of the colonies; and
5 screening the colonies to identify colonies with active glucose oxidase after
6 altering the environment of the colonies.

1 27. A method for formulating an enzyme according to claim 26, wherein the
2 organism is selected from a group consisting of *Aspergillus Niger*, *Penicillium funiculosum*,
3 *Saccharomyces cerevisiae*, and *Escherichia Coli*.

1 28. A method for formulating an enzyme according to claim 26, wherein altering the
2 environment of the colonies comprises introducing peroxide to the colonies.

1 29. A method for formulating an enzyme according to claim 26, wherein screening
2 the colonies to identify colonies with active glucose oxidase comprises employing a substance
3 that changes color in the presence of active glucose oxidase.

1 30. A method for formulating an enzyme according to claim 29, wherein the
2 substance is leuco-crystal-violet.

1 31. A method for formulating an enzyme according to claim 30, wherein screening
2 the colonies to identify colonies with active glucose oxidase comprises checking for
3 fluorescence.

1 32. A method for formulating an enzyme according to claim 26, wherein the method
2 further comprises testing the colonies with active glucose oxidase for functionality after
3 screening the colonies to identify colonies with active glucose oxidase.

1 33. A method for formulating an enzyme according to claim 32, wherein the method
2 further comprises continuing to alter the environments of the colonies until the colonies with
3 active glucose oxidase are of a suitable number to proceed with testing the colonies with active
4 glucose oxidase for functionality.

1 34. A method for formulating an enzyme according to claim 32, wherein testing the
2 colonies with active glucose oxidase for functionality comprises employing glucose oxidase
3 from the colonies in sensors.

1 35. A method for formulating an enzyme according to claim 32, wherein testing the
2 colonies with active glucose oxidase for functionality comprises:

3 extracting glucose oxidase from the colonies;
4 immobilizing the glucose oxidase after extracting the glucose oxidase from the
5 colonies;
6 placing the immobilized glucose oxidase in a sensor; and
7 testing the sensor.

1 36. A method for formulating an enzyme according to claim 35, wherein extracting
2 glucose oxidase from the colonies comprises employing an ionic column to extract glucose
3 oxidase from the colonies.

1 37. A method for formulating an enzyme according to claim 35, wherein extracting
2 glucose oxidase from the colonies comprises:
3 removing the glucose oxidase from the colonies;
4 purifying the glucose oxidase; and
5 characterizing the glucose oxidase.

1 38. A method for formulating an enzyme according to claim 37, wherein removing
2 the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell
3 components.

1 39. A method for formulating an enzyme according to claim 38, wherein removing
2 the glucose oxidase from the colonies further comprises fractionating the cell components
3 employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

1 40. A method for formulating an enzyme according to claim 37, wherein removing
2 the glucose oxidase from the colonies comprises disrupting the colonies into cell components via
3 sonication.

1 41. A method for formulating an enzyme according to claim 40, wherein removing
2 the glucose oxidase from the colonies further comprises fractionating the cell components
3 employing centrifugation and differential solubility after disrupting the colonies via sonication.

1 42. A method for formulating an enzyme according to claim 37, wherein purifying
2 the glucose oxidase comprises purifying the glucose oxidase by employing chromatography
3 methods.

1 43. An enzyme formulated according to the method of claim 26.

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